The Human Papillomavirus Type 11 Upstream Regulatory Region Triggers Hair-Follicle-Specific Gene Expression in Transgenic Mice

J. Schenkel,†‡ H. Gaissert,* E. E. Protopapa,§ H. Weiher,‡[1] L. Gissmann,* and A. Alonso*

*Deutsches Krebsforschungszentrum, Heidelberg, Germany; †II. Physiologisches Institut der Universität, Heidelberg, Germany; §Department of Pharmacy, University of Athens and Technological Educational Institution, Athens, Greece; ‡Institut fur Genetik, Universitat/Forschungszentrum, Karlsruhe, Germany

We have generated transgenic mice carrying the URR of the human papillomavirus type 11 ligated in front of the *Escherichia coli* β-galactosidase coding region sequence. Using X-Gal staining to demonstrate β-galactosidase production, we observed a hair-specific transcription of the reporter gene. This transcription was limited to the epithelial cells of the hair bulge region. The transgene was developmentally regulated, as no LacZ staining was demonstrated during embryogenesis and specific staining was first observed after birth. Surprisingly, dexamethasone and ultraviolet B, but not phorbol myristate acetate or progesterone treatment of the animals resulted in an increase in number and intensity of hair follicles expressing the reporter gene. *Key words: promoter/skin/transcription. J Invest Dermatol 112:893–898, 1999*

Human papillomaviruses (HPV) are epitheliotropic small tumor viruses with a circular double-stranded DNA genome of ≈8 kb. More than 90 different HPV types have so far been identified, some of which are preferentially associated with benign lesions (low-risk type, such as types 1, 6, and 11) whereas others are found mainly in association with malignant tumors (high-risk type, such as types 16, 18, and 33). The low-risk type HPV11 is described to infect the mucosa of the organs of the genital tract and the perianal area, although viral sequences have also been detected in cervical neoplasias and laryngeal papillomas (Dartmann *et al*, 1986; Henderson *et al*, 1987; Guillou *et al*, 1991; Shen *et al*, 1996).

Initiation of transcription of all HPV early genes takes place preferentially at the upstream regulatory region or URR (an E7 internal promoter has also been described), which contains binding elements for cellular as well as viral regulatory proteins (Chan *et al*, 1989; Thierry *et al*, 1992; Thierry, 1993). As in the case with other HPV types, the URR sequence of HPV11 contains promoter and enhancer properties (Auborn *et al*, 1989; Auborn and Steinberg, 1991; Hoppe-Seyler and Butz, 1994). The promoter region has been found to enclose two well defined functional regions, CEI and CEII, which are responsible for a cell-specific expression (Dollard *et al*, 1993). In addition, it appears that HPV11 lacks the octamer-binding site found in the high-risk types (Morris *et al*, 1993) and it has been shown that C/EBPβ transcription factor acts as a negative regulator in HPV11 (Wang *et al*, 1996). Putative binding sites for AP-1, SP1, and NF1 have also been described (Dollard *et al*, 1993). Interestingly, viral transcription in cultured cells derived from laryngeal papillomas carrying HPV11 sequences can be modulated by various agents such as calcium or retinoic acid (DiLorenzo and Steinberg, 1995). Thus, although the general structure of the URR follows the rules of all other HPV types, specific differences in this region suggest that HPV11 regulates transcription by mechanisms different to the known high-risk HPV types.

Little is known about the control mechanisms regulating the transcription of HPV sequences *in vivo*. To date, only mice transgenic for high-risk HPV-URR sequences have been investigated and no data have been published concerning the low-risk types (Choo *et al*, 1992; Cid *et al*, 1993; Chen *et al*, 1995; Michelin *et al*, 1997). The aim of this work, therefore, was to investigate the transcriptional regulation of the HPV11-URR sequences in transgenic mice. Animals were generated carrying the *LacZ* coding sequence under the control of the URR region of the HPV type 11. Analysis of the transgenic animals obtained demonstrated a tight and specific transcriptional pattern of the transgene to the hair bulge epithelial cells. Most interestingly, HPV11-URR-dependent gene expression can be modulated by treatment with glucocorticoids or ultraviolet (UV) light, making these animals a valuable tool to study the effects of environmental noxae on the transcriptional characteristics of low-risk HPV types.

MATERIALS AND METHODS

Cloning procedure The bacterial *lacZ* gene was cloned into the *Hind*III/*Hin*cII site of the BlueScript KS-vector. The *Bam*HI/*Bsm*I URR fragment was isolated from a plasmid containing the complete HPV11 genome cloned into pBR322 and introduced into the *Xba*I site of the BlueScript-*lacZ* vector by blunt end ligation. The DNA of the resulting recombinant HPV11-URR/β-gal plasmid was sequenced to verify orientation of the URR (**Fig 1**).

Generation and identification of transgenic mice The *Not*I/*Apa*I fragment of the recombinant plasmid, containing the HPV11-URR and β galactosidase reporter gene (**Fig 1**), was isolated by agarose gel electrophoresis, purified, and used for microinjection into pronuclei of zygotes

Manuscript received July 28, 1998; revised January 26, 1999; accepted for publication February 17, 1999.

Reprint requests to: Dr. A. Alonso, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, 69120 Heidelberg, Germany.

[1]Present address: Institut fur Diabetesforschung, Munchen, Germany

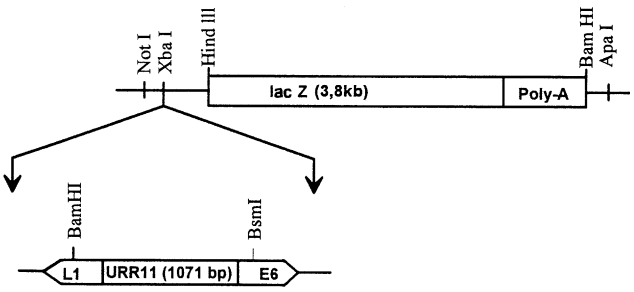

Figure 1. Schematic representation of the recombinant used for the production of transgenic animals. The *Bam*HI–*Bsm*I fragment of the HPV11 DNA (1071 base pairs) was cloned in front of the bacterial *lacZ* gene (3.8 kb) in the plasmid pBluescript KS. The 5 kb long *Not*I–*Apa*I fragment was excised from the plasmid, isolated and used for the generation of transgenic mice.

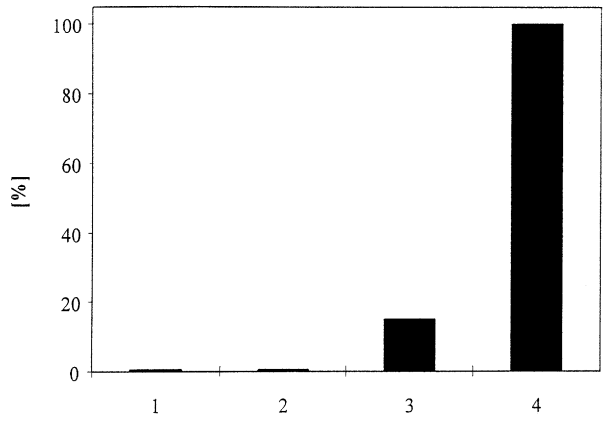

Figure 2. Transfection of the recombinant in HaCaT cells. Dishes (10 cm) of HaCaT cells were grown to 80% confluence in DMEM and transfected with 20 μg of recombinant DNA as described (Nead and McCance, 1995). In parallel, control dishes were transfected with a recombinant plasmid containing the *LacZ* gene under the control of the CMV promoter. After 48 h, extracts were prepared and the β-galactosidase activity was measured using CPRG as chromogen. The values of the CMV-*LacZ* (*lane 4*) were taken as 100% and the activity of the URR-*LacZ* recombinant (*lane 3*) was calculated accordingly. *Lane 1* represents a water control and *lane 2* is the β-galactosidase activity of cell extracts transfected with a promoterless *LacZ* gene.

generated from F_1 hybrid female mice (C57Bl6xDBA/2) mated to DBA/2 males. Surviving one- or two-cell embryos were transferred into the oviducts of pseudopregnant foster mice according to published methods (Hogan *et al*, 1986).

Tail biopsies were taken and digested overnight in 800 μl of 'tail-buffer' [50 mM Tris pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% (wt/vol) sodium dodecyl sulfate, 0.5 mg per ml proteinase K] at 55°C. After protein precipitation with salt, the DNA was precipitated with isopropanol, washed, digested with restriction enzymes, and separated on agarose gels. Southern blots were hybridized at 65°C with a ^{32}P-radiolabeled URR fragment. Washing steps were performed at 65°C for 15 min with 0.2 × sodium citrate/chloride buffer and 0.2% sodium dodecyl sulfate.

Treatment of animals To avoid an irritation of the skin, the back of the mice was shaved using an electrical shaver 24 h prior to treatment. Dexamethasone was dissolved in acetone at a concentration of 2.5 mg per ml and 50 μl of this solution were spotted on to the back of the mice. The same amount of solvent was spotted elsewhere onto the same animal as control. Animals were killed 6, 24, 48, and 72 h after dexamethasone treatment and biopsies of the spots were taken, frozen and used for X-Gal staining. For UVB treatment (UVB lamp of a wavelength 280–320 nm) narcotized animals were covered with a sheet leaving only two windows of 1 cm^2 uncovered. Irradiation was at a dose of 2 W per cm^2 At 8, 16, 24, and 48 h after irradiation, skin biopsies were taken for analysis. For quantitation, at least 100 follicles were counted in every slide and at least three slides were counted per transgenic mouse. A total of 10 animals was used for each experiment and each experiment was repeated at least two times.

Treatment with proteolytic enzymes was performed by iontophoresis or via lipid vehicles as described (Protopapa, 1997); briefly, anesthetized mice were depilated using warm wax and proteolytic enzymes (1 mg per ml of chymotrypsin and papain) were applied to the skin using an electrode at 2 mA for 2 min. Alternatively, the enzymes were packaged into liposomes and rubbed twice within 48 h on to the depilated skin of mice for 8 min.

X-Gal staining Mice were killed and the organs were dipped into cooled isopentane, frozen, and conserved in liquid nitrogen. Cryosections of 6–10 μm were performed in a cryostat at −20°C and transferred to clean, 3-aminopropyltriethoxysilane dissolved in acetone (Sigma, Munich, Germany) coated glass slides. The samples were fixed for 5 min at 4°C in 2% paraformaldehyde, 0.2% glutaraldehyde, 0.02% NP-40, 0.01% sodium dodecyl sulfate in phosphate-buffered saline, and then washed in phosphate-buffered saline again. The slides were covered with 600 μl reaction mix (1 mg per ml X-Gal [5-bromo-4-chloro-3 indolyl-β-D-galctopyranoside], 5 mM $K_3Fe[CN]_6$, 5 mM $K_4Fe[CN]_6$ and 2 mM $MgCl_2$ in phosphate-buffered saline [0.14 M NaCl, 0.003 M KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4]) in the dark, in a humidity chamber overnight at 37°C and then rinsed in phosphate-buffered saline. Slides were counterstained with hematoxylin–eosin.

Other methods Transfections were performed in HaCaT cells using poly L-ornithine as described (Nead and McCance, 1995). Forty-eight hours after addition of the DNA, cell extracts were prepared and the β-galactosidase activity was measured using chlorophenol red-β-D-galacto-pyranoside as chromogen. For RNA isolation, deep frozen organs were pulverized in a mortar under liquid nitrogen and the RNA was extracted using the RNeasy kit (Quiagen, Hilden Germany) according to the manufacturers instructions. Reverse transcriptase–polymerase chain reaction (PCR) was performed with the Access RT–PCR System (Promega). The forward and reverse PCR primers used were ^{3470}GAGATTGGCGTAA-GTGAAGC^{3490} and ^{3989}GTTGACTGTAGCGGCTGATG^{3970}, respectively. The size of the expected amplification product was 520 bp.

RESULTS

Expression of the transgene in transfected human epithelial cells To test whether or not the HPV11-URR construct is active in transcription, human HaCaT cells were transiently transfected with the recombinant HPV11-URR/β-gal plasmid (see *Materials and Methods* and **Fig 1**) and the amount of expressed β-galactosidase was calculated. HaCaT is an immortal keratinocyte cell line isolated from adult skin. As shown in **Fig 2**, the HPV-URR was indeed able to drive transcription of the reporter gene in HaCaT cells, albeit at a low level when compared with recombinants carrying the CMV promoter. Thus, the HPV11-URR construct was used to generate transgenic mice.

Generation of transgenic animals For the generation of the transgenic mice, the HPV11-URR/β-gal *Not*I/*Apa*I fragment was isolated from the vector (see **Fig 1**), purified and microinjected into pronuclei. From a total of 150 embryos microinjected, 80 were implanted into foster mothers and a total of 14 transgenic animals were obtained. Four lines (1697, 1702, 1704, and 1747) were used for further analysis, because these mice were able to express the transgene *in vivo* and to transmit it to their offsprings. The other 10 founder lines did not express the transgene or did not transmit it to their offspring properly. DNA isolated from these animals was restriction cut with different enzymes and the relative number of incorporated transgene copies determined. As shown in **Fig 3**, three of the lines (1697, 1704, and 1747) contained a similar number of copies, whereas a fourth one (line 1702) carried five to 10 times more copies. Using the corresponding standards we calculated for the first three lines a number of less than 20 copies per haploid genome. In addition, further restriction analyses of the DNA demonstrated a head-to-tail concatemeric structure of the integrated copies (not shown). Transgene line 1755 was not analyzed further as the transgene was not transmitted properly during subsequent breeding.

Expression of the transgene To analyze whether the HPV11-URR was able to support expression of the transgene in skin

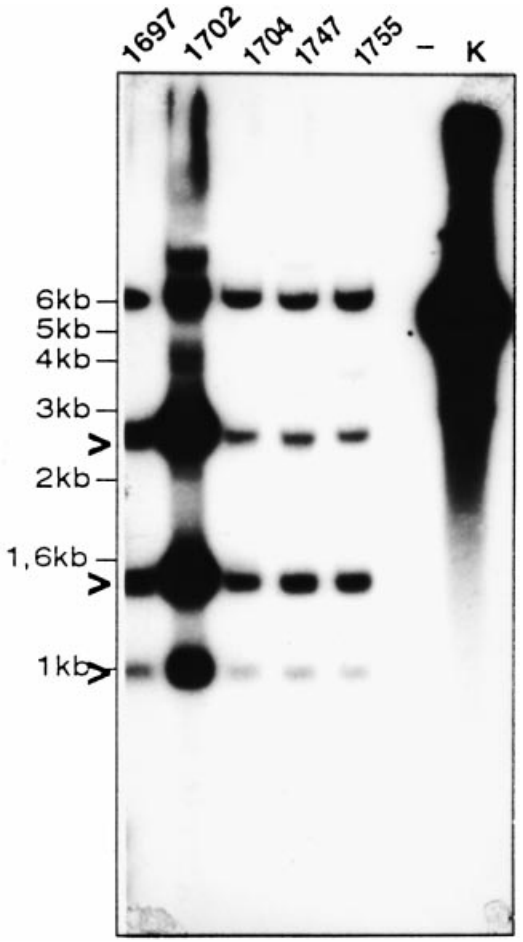

Figure 3. Southern blot analysis of the founders 1697, 1702, 1704, and 1747. Mouse tail DNA (10 μg) was digested with *Eco*RV, separated on 0.7% agarose gels, blotted on to nylon membranes, and hybridized with a radioactive labeled URR-*LacZ* DNA probe. Three fragments of a length of 0.99, 1.5, and 2.4 kb were obtained, corresponding to the fragments appearing after digestion of the original recombinant with *Eco*RV. This pattern, together with results from other digestions, suggests a head-to-tail organization of the recombinant DNA into the transgenic mouse genome.

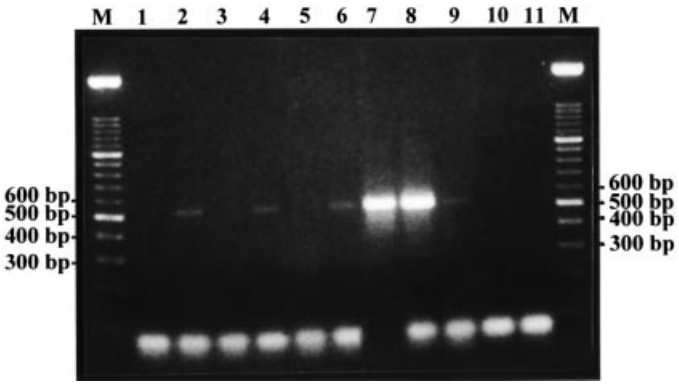

Figure 4. Reverse transcriptase–PCR analysis of skin mRNA. RNA was extracted from skin of the transgenic lines 1697 (*lanes 1* and *2*) 1704 (*lanes 3* and *4*), and 1747 (*lanes 5* and *6*). In *lanes 1*, *3*, and *5*, reverse transcriptase was omitted. *Lanes 7–9* show PCR products using different amounts of the starting recombinant DNA as template. *Lanes 10* and *11* are negative controls without RNA and without Taq polymerase, respectively.

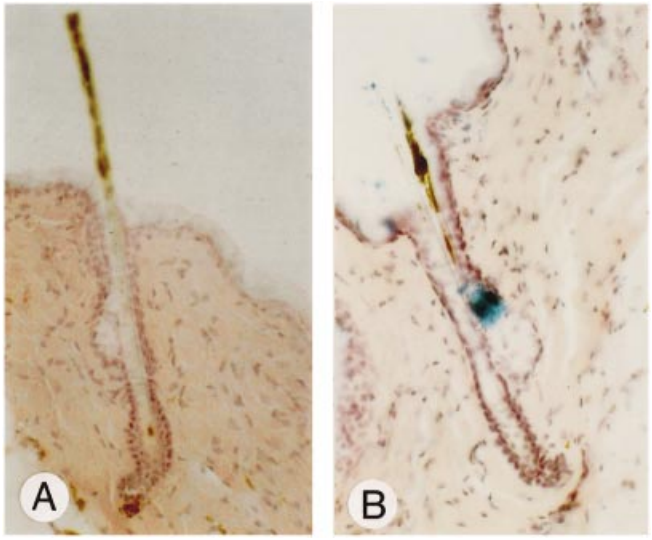

Figure 5. Histochemical detection of β-galactosidase in the skin of the transgenic mice. Cryosections 8 μm thick of the dorsal skin of transgenic mice were prepared, fixed and stained with X-Gal overnight. Slides were counterstained with hematoxylin–eosin. (*A*) skin of a mouse unable to transmit the transgene; (*B*) skin of a mouse of the transgenic line 1704.

epithelia, RNA from the skin of different transgenic mice was isolated and reverse transcriptase–PCR was performed using the primers described in *Materials and Methods*. Indeed a fragment of the expected size, 520 base pairs, was amplified (**Fig 4**) and no amplified fragment was observed in the reaction performed without reverse transcriptase, demonstrating that the amplified band obtained did not originate from contaminating DNA. Interestingly, although the reverse transcriptase–PCR methodology used does not allow accurate transcript quantitation, the similar intensity of the amplified bands shown in **Fig 4** suggests that transcription of the transgene was similar in all animals tested.

Expression of β-galactosidase was analyzed in frozen sections of various tissues isolated from the founder lines 1697, 1702, 1704, and 1747, by histochemical staining with X-Gal. No expression of the transgene was detected in any of the analyzed tissues or organs known to harbour papillomaviruses in human lesions, such as uterus, cervix, esophagus, tongue, vagina, testis, and penis, or in those tissues known to be free of viral infection, such as stomach, spleen, liver, kidney, ovary, and brain (data not shown). Surprisingly, strong expression was found in the bulge region of the hair follicles. In most of the slides, staining was confined to the epithelial cells located between the sebaceous gland and the outer root sheath of the hair (**Figs 5*B*** and **6*B***). In some sections, a slight pale blue staining was found distributed evenly over the sebaceous gland, but we interpret this as the result of X-Gal diffusion from the neighboring cells during the staining procedure (see next paragraph). No staining was observed in control animals nor in animals shown not to transmit the transgene to their offsprings (**Figs 5*A***, *top panel* and **6*A***). Furthermore, expression was limited to a fraction of the total number of follicles, varying between 7 and 14% in the different transgenic lines obtained.

To analyze whether expression of the transgene was developmentally regulated, complete embryo sections were prepared from embryos between days E12 and E19. No X-Gal staining was detected in any of the transgenic lines at any of the embryonic stages investigated (**Fig 6*C*** and data not shown). *LacZ* staining was first found in 18 d old adults, at which time the growth of hair still occurs synchronously (Handjisky *et al*, 1994). In addition, the expression of the transgene in the adults was independent of age and sex during the subsequent 2 y period of observation. The fact that the expression of the transgene in all four founder lines showed the same pattern of expression would indicate that the expression was HPV11-URR specific and not due to a position effect of the integrated transgene in the host genome.

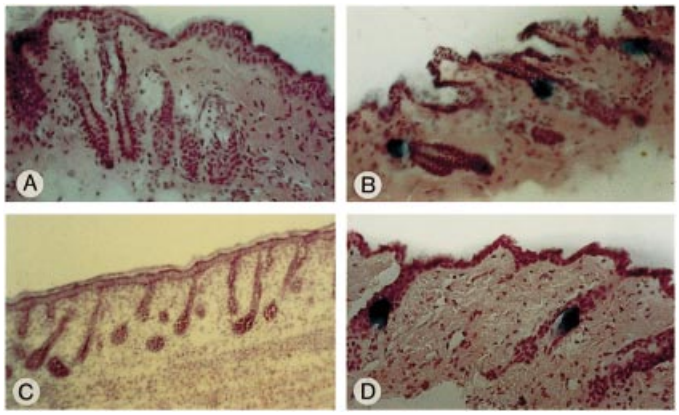

Figure 6. Characterization of X-Gal staining cells. Cryosections were prepared and stained as described in **Fig 5**. (*A*) Staining of the dorsal skin of a male (line 1775, nontransgenic) showing no X-Gal stain. (*B*) Skin of a 2 mo old transgenic male (line 1697) showing staining over the hair bulge region. (*C*) skin of an embryo at day 19 of embryogenesis. (*D*) X-Gal staining of the skin of a mouse (line 1697) after application of proteolytic enzymes by iontophoresis. Magnification, × 450.

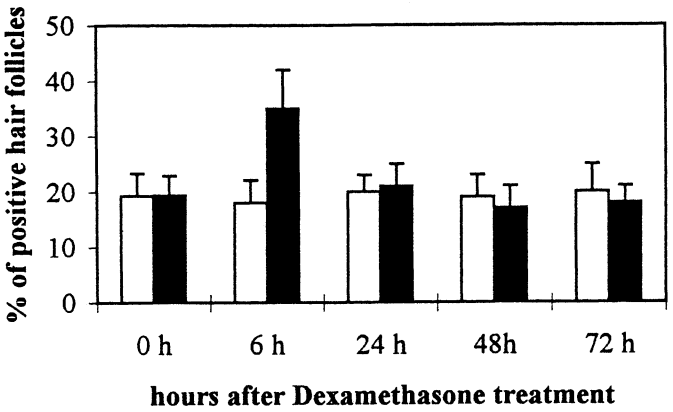

Figure 7. Time course of dexamethasone treatment. Dexamethasone was applied on to the back of transgenic mice (lines 1704 and 1742). The solvent acetone was applied elsewhere on the same animals as negative control. The animals were killed 0, 6, 24, 48, and 72 h later cryosections were stained with X-Gal staining. The percentage of stained hair follicles out of all hair follicles is shown. The values for acetone-treated animals are shown in open bars, those for transgenic animals in black bars. Quantitation was performed as described in *Materials and Methods*.

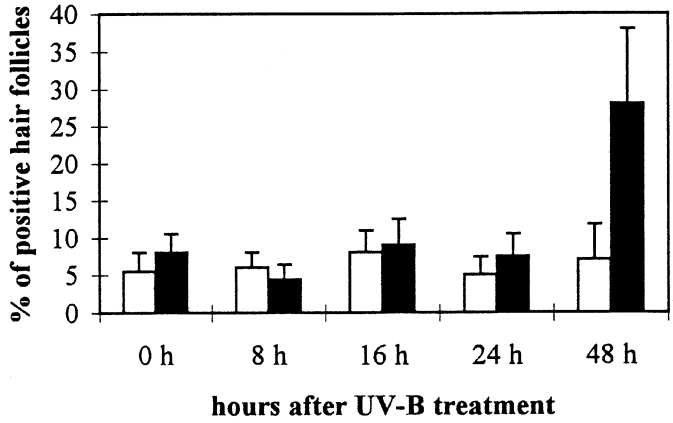

Figure 8. Time course of UVB treatment. Transgenic animals (the same lines as in **Fig 6**) were irradiated at the doses described in *Materials and Methods*. Controls were biopsies of the same animals from skin parts covered during irradiation. The animals were killed 0, 8, 16, 24, and 48 h after irradiation and cryosections were prepared from the skin and stained with X-Gal. The percentage of stained hair follicles out of all hair follicles is shown. The open bars show the values for untreated animals; UVB-treated transgenic mice are shown in black bars.

Localization of transgene expression As described above, the reporter gene is only expressed in cells of the hair follicles leaving the interfollicular epidermis free of staining. This is extremely interesting as it has been suggested that the stem cells of the skin are localized to this region and that, these cells are responsible for hair growth as well as for the regeneration of the interfollicular epithelium. Immunofluorescence studies, using β-galactosidase and keratin 19 antibodies, thought to be specific for the putative stem cells (Michel *et al*, 1996), prove inconclusive. We therefore attempted to further characterize the expressing cells type by the iontophoretic protocol (Protopapa, 1997).

The proteases papain and chymotrypsin are transported by iontophoresis or by liposomes into the hair bulge. The action of these enzymes leads to the destruction of the intercellular keratinocyte structure in the stratum spinosum resulting in a withdrawal of the keratin layer from the stratum granulosum and in the proteolysis of epithelial stem cells. In contrast, the cells of the sebaceous glands remain protected from the exogenous protease activity by the secreted sebum (Protopapa, 1997). Thus, in the case of an expression of the reporter gene in epithelial cells, one would expect a change in X-Gal staining localization following treatment with chymotrypsin and papain. Whereas in the case of expression of the reporter gene in the cells of the sebaceous gland, one would expect the protease treatment to have no influence on the X-Gal staining. As shown in **Fig 6(*D*)**, following chymotrypsin and papain treatment, the β-galactosidase activity was detected only in the lumen of the hair follicle and no longer in its previous place (**Fig 6*B***). We therefore conclude that expression of the transgene takes place preferentially, if not exclusively, in the epithelial cells surrounding the upper part of the sebaceous gland.

Transcriptional control of the transgene *in vivo* The URR of the HPV11, as used for the generation of the transgenic animals, contains putative GRE and AP-1 motifs suggesting that promoter activity can be controlled by corticoids or agents regulating activity of the AP-1 protein, such as phorbol esters or UV light. Thus, transgenic animals were topically treated either with dexamethasone or with phorbol myristate acetate (PMA), and the number of hair follicles expressing the transgene at different times after application was calculated. Whereas dexamethasone elicited an increase in the number of positive follicles 6 h after application, no effect was observed in PMA-treated animals (**Fig 7** and data not shown). Further, the increase in *LacZ* expression in dexamethasone-treated animals disappears 24 h after treatment (**Fig 7**). Thus dexamethasone appears to modulate transcription of the gene in a precise manner, similar to that described for mice carrying the HPV18 URR region cloned in front of the *LacZ* coding sequence (Cid *et al*, 1993). In addition, treatment of the animals with 0.5 mg progesterone did not result in any change in number or intensity of stained hair follicles (data not shown), in accordance with the observation that expression of the transgene was sex- and estrus-independent.

The UVB radiation experiments showed no change in the expression pattern until 48 h after irradiation. At 48 h after irradiation a strong increase in the number of *LacZ*-positive follicles was observed (**Fig 8**). We also noticed that, at this time there was an observable increase in expression of the transgene in the follicles, based on optical observation of the stained slides (a precise quantitation of the β-galactosidase was not possible for technical reasons). At 72 h after irradiation, staining declined to values similar to the 24 h time-point. Thus, dexamethasone and UVB are able to modulate the transcriptional characteristics of the HPV11-URR under *in vivo* conditions.

DISCUSSION

We have generated transgenic mice carrying the URR of the low-risk type HPV11 in front of the β-galactosidase as a reporter gene

and have analyzed the transcriptional characteristics of the transgene. Four founder lines were found to express the transgene and to transmit it to their descendants, three of which possessed a similar number of integrated copies and transcribed the gene to similar levels.

The finding that our transgenic mice expressed the transgene exclusively in the hair follicles was unexpected. No *LacZ* staining was observed in those tissues known to be a target for HPV11 infection, nor in other tissues refractory to viral infection. These results sharply contrast with those obtained when the HPV18-URR was used for the generation of transgenic mice (Choo *et al*, 1992; Cid *et al*, 1993; Chen *et al*, 1995). Although contradictory results have been published, *LacZ* expression in HPV18-URR transgenic mice appears to be either limited to the epithelial cells or widely distributed among all organs (Cid *et al*, 1993; Michelin *et al*, 1997). The reasons for the differences between types 11 and 18 are unknown. These differences cannot rely in the use of different mice strains to produce the transgenic animals, as in both experiments the same strain, as well as the same starting reporter plasmid, were used. Furthermore, although the URR of both types are similar (both contain putative binding motifs for AP-1, SP1, NF1, or C/EBPβ), and both viruses replicate in human epithelial cells, slight differences in the redundancy or activity of some elements might be responsible for the differences in specificity observed (Morris *et al*, 1993). In addition, mechanisms linked to the natural state of the virus (predominantly episomal in the case of HPV11 or integrated in the case of HPV18) may further play a part in establishing cell specificity and transcriptional efficiency.

Whereas, in humans HPV11 expression is found preferentially in interfollicular keratinocytes, in mice *LacZ* staining is localized exclusively to the hair epithelium. The reasons for this difference are unknown but it may well be that human keratinocytes contain all the specific factors necessary to complete HPV11-specific transcription, factors which would be absent in mouse interfollicular epithelium. Histologic examination of skin sections of the transgenic mice allowed the localization of the β-galactosidase expression to the epithelial cells associated with the sebaceous glands. The light blue color sometimes observed in the glandular cells was interpreted to be due to color diffusion from the epithelial cells during X-Gal staining. This assumption is based on several facts: we always found clear staining in the epithelial cells and only a pale bluish staining in the sebaceous glands. Further, the iontophoretic experiments demonstrate that the X-Gal staining, remaining after treatment, is exclusively found in the interior of the hair follicle and not in the position corresponding to the sebaceous glands. This is the position the remaining bulge cells adopt after papain/chymotrypsin treatment of the skin by iontophoresis (Protopapa, 1997).

It is interesting to note that the epithelial bulge cells are probably the primary target cells of papillomavirus infection in cottontail rabbits (Schmitt *et al*, 1996). There is evidence that HPV persists in hair follicles, although the biologic significance of this finding, as well as the precise localization are currently unknown (J. ter Schegget, personal communication and our unpublished results). It remains open as to whether the cells expressing the transgene in our animals correspond to those cells found to harbor HPV sequences in human infections or if the cells are intact skin stem cells. In any case, we did not unequivocally observe the presence of keratin 19 (a potential marker for keratinocytes stem cells, Michel *et al*, 1996) in the β-galactosidase positive cells. Further, low-risk and high-risk HPV sequences have also been found in sebaceous gland carcinomas of the eyelid and vagina but not in normal keratinocytes (Hayashi *et al*, 1994; Carlson *et al*, 1996). In this context it is interesting that mouse skin develops more tumors when the carcinogen is applied during early anagen phase, implicating therefore the bulge cells as potential precursors of some skin tumors (Miller *et al*, 1993). Thus, we believe that our transgenic animals express the transgene solely in the epithelial cells surrounding the sebaceous gland of the hair and that this expression can be regulated by external stimuli.

Expression from the URR appears to be developmentally regulated, as no *LacZ* staining was found in embryos, even at day 18 of pregnancy, when the hair structure is already well developed. It is noteworthy that in juvenile mice a stronger expression of the transgene was observed, perhaps depending on the hair growth phase at this age (Miller *et al*, 1993). This, and the fact that all other organs analyzed in adults were negative, suggests a tight regulation of the URR by factors present only in the keratinocytes of the hair follicle. These factors presumably confer cell specificity to the transgene. In HPV11 it seems well established that the CEI region of the URR is responsible for cell specificity in *in vitro* transfection experiments (Dollard *et al*, 1993).

Although little is known about the mechanisms responsible for HPV specific gene expression, it would appear that these mechanisms are related to transcription rather than to infection. The description of a putative HPV receptor in several cell types, some of them of nonepithelial origin, supports this possibility (Evander *et al*, 1997). As HPV is mainly found as an episome in infected cells, it is possible that tight regulation is accounted for by mechanisms related to the episomal stage and that upon integration into the host genome these mechanisms are totally or partially abolished. In any case, it seems that viral integration, at least HPV16 in SiHa cells, does not instigate large chromatin rearrangements at the integration locus (Bauer-Hofmann *et al*, 1996).

The regulatory properties of the URR appear to be conserved after incorporation into the mouse genome, as treatment with dexamethasone or UVB clearly modulate gene expression (see *Results*). In both HPV18-URR and HPV11-URR transgenic mice, treatment with dexamethasone triggers increased expression of the *LacZ* gene in the expressing tissues. In both cases increased expression occurs 6 h after treatment, suggesting that the mechanisms responsible for modulation are similar in both HPV types.

Interestingly, the tumor promoter PMA fails to modulate transcription via the URR. Our results show that, even 48 h after treatment, no increase in X-Gal staining could be observed. This is in contrast to the increase observed after irradiation with UVB light. As both PMA and UVB probably function by increasing transcription of the AP-1 genes, one might expect that both agents would produce a similar increase in transgene transcription. This was clearly not the case in our experiments. Interestingly, we found a large increase in the amount of AP-1 protein 48 h after skin irradiation of our transgenic animals, declining thereafter (our unpublished results). These results are in agreement with those published before, which also described an increased AP-1 expression in transgenic mice after UVB treatment (Huang *et al*, 1997). Thus it seems that protein kinase C activation by PMA and subsequent transcription of AP-1 proteins are not enough to modulate activation of the HPV11 promoter, and that other, additional effects are necessary to activate *in vivo* transcription of the HPV11-URR.

Taken together, our results demonstrate that, in our transgenic animals, HPV11-URR drives transcription exclusively in the epithelial cells associated with the sebaceous glands and that this transcription is tightly regulated. The use of the promoter region described here in conjunction with genes potentially involved in epithelial proliferation/differentiation should provide an invaluable tool for the analysis of hair development.

REFERENCES

Auborn KJ, Steinberg BM: A key DNA–protein interaction determines the function of the 5′-URR enhancer in human papillomavirus type 11. *Virology* 181:132–138, 1991

Auborn KJ, Galli RL, DiLorenzo TP, Steinberg BM: Identification of DNA–protein interactions and enhancer activity at the 5′end of the upstream regulatory region in human papillomavirus type 11. *Virology* 170:123–130, 1989

Bauer-Hofmann R, Borghouts C, Auvinen E, Bourda E, Rosl F, Alonso A: Genomic cloning and characterization of the non-occupied allele corresponding to the integration site of human papillomavirus type 16 DNA in the cervical cell line SiHa. *Virology* 217:33–41, 1996

Carlson JW, McGlennen RC, Gomez R, Longbella C, Carter J, Carson LF: Sebaceous carcinoma of the vulva: a case report and review of the literature. *Gynecol Oncol* 60:489–491, 1996

Chan WK, Klock G, Bernard HU: Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. *J Virol* 63:3261–3269, 1989

Chen CM, Choo KB, Cheng WT: Frequent deletions and sequence aberrations at the transgene junctions of transgenic mice carrying the papillomavirus regulatory and the SV40 Tag gene sequences. *Transgenic Res* 4:52–59, 1995

Choo KB, Chong KY, Liew LN, Hsu HC, Cheng WT: Unregulated and basal transcriptional activities of the regulatory sequences of the type 18 human papillomavirus genome in transgenic mice. *Virology* 188:378–383, 1992

Cid AP, Auwarakul P, Garcia-Carranca A, Ovseiovich R, Gaissert H, Gissmann L: Cell-type-specific activity of the human papillomavirus type 18 upstream regulatory region in transgenic mice and its modulation by tetradecanoyl phorbol acetate and glucocorticoids. *J Virol* 67:6742–6752, 1993

Dartmann K, Schwarz E, Gissmann L, zur Hausen H: The nucleotide sequence and genome organization of human papillomavirus type 11. *Virology* 151:121–130, 1986

DiLorenzo TP, Steinberg BM: Differential regulation of human papillomavirus type 6 and 11 early promoters in cultured cells derived from laryngeal papillomas. *J Virol* 9:6865–6872, 1995

Dollard SC, Broker TR, Chow LT: Regulation of the human papillomavirus type 11, E6 promoter by viral and host transcription factors in primary human keratinocytes. *J Virol* 7:1721–1726, 1993

Evander M, Frazer ICH, Payne E, Qi YM, Hengst K, McMillan NA: Identification of the α6 integrin as a candidate receptor for papillomaviruses. *J Virol* 71:2449–2456, 1997

Guillou L, Sahli R, Chaubert P, Monnier P, Cuttat JF, Costa J: Squamous cell carcinoma of the lung in a nonsmoking, nonirradiated patient with juvenile laryngotracheal papillomatosis. Evidence of human papillomavirus-11 DNA in both carcinoma and papillomas. *Am J Surg Pathol* 5:891–898, 1991

Handjisky BK, Eichmuller S, Hofmann U, Czanetzki BM, Paus R: Alkaline phosphatase activity and localization during the murine hair cycle. *Br J Dermatol* 131:303–310, 1994

Hayashi N, Furihata M, Ohtsuki Y, Ueno H: Search for accumulation of p53 protein and detection of human papillomavirus genomes in sebaceous gland carcinoma of the eyelid. *Virchows Arch* 424:503–509, 1994

Henderson BR, Thompson CH, Rose BR, Cossart YE, Morris BJ: Detection of specific types of human papillomavirus in cervical scrapes, anal scrapes, and anogenital biopsies by hybridization. *J Med Virol* 21:381–393, 1987

Hogan B, Constantini F, Lacy E: *Manipulating the Mouse Embryo: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1986

Hoppe-Seyler F, Butz K: Cellular control of human papillomavirus oncogene transcription. *Carcinogenesis* 10:134–141, 1994

Huang C, Ma WY, Hanenberger D, Cleary MP, Bowden GT, Dong Z: Inhibition of ultraviolet B-induced activator protein-1 (AP-1) activity by aspirin in AP-1-luciferase transgenic mice. *J Biol Chem* 272:26325–26331, 1997

Michel M, Torok N, Godbout MJ, Lussier M, Gaudreau P, Royal A, Germain L: Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. *J Cell Sci* 109:1017–1028, 1996

Michelin D, Gissmann L, Street D, *et al*: Regulation of human papillomavirus type 18 *in vivo*: effects of estrogen and progesterone in transgenic mice. *Gynecol Oncol* 66:212–218, 1997

Miller SJ, Wie ZG, Wilson C, Dzubow L, Sun TT, Lavker RM: Mouse skin is particularly susceptible to tumor initiation during early anagen of the hair cycle: possible involvement of hair follicle stem cells. *J Invest Dermatol* 101:591–594, 1993

Morris PJ, Dent CL, Ring CJ, Latchman DS: The octamer binding site in the HPV16 regulatory region produces opposite effects on gene expression in cervical and non-cervical cells. *Nucleic Acids Res* 21:1019–1023, 1993

Nead MA, McCance DJ: Poly-L-ornithine-mediated transfection of human keratinocytes. *J Invest Dermatol* 105:668–671, 1995

Protopapa E: Elements of enzymic depilation. Athens: Omvros edn, 1997

Schmitt A, Rochat A, Zeltner R, Borenstein L, Barrandon Y, Wettstein FO, Iftner T: The primary target cells of the high-risk cottontail rabbit papillomavirus colocalize with hair follicle stem cells. *J Virol* 70:1912–1922, 1996

Shen J, Tate JE, Crum CP, Goodman ML: Prevalence of human papillomaviruses (HPV) in benign and malignant tumors of the upper respiratory tract. *Mod Pathol* 9:15–20, 1996

Thierry F: Proteins involved in the control of HPV transcription. *Papillomavirus Rep* 4:27–32, 1993

Thierry F, Spyrou G, Yaniv M, Howley P: Two AP1 sites binding JunB are essential for human papillomavirus type 18 transcription in keratinocytes. *J Virol* 66:3740–3748, 1992

Wang H, Liu K, Yuan F, Berdishevsky L, Taichman LB, Aunorn K: C/EBPβ is a negative regulator of human papillomavirus type 11 in keratinocytes. *J Virol* 70:4839–4844, 1996